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Observations on the Morphology and Taxonomy of *Phycopeltis hawaiiensis* King (Chroolepidaceae)[1]

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ABSTRACT: Observations with light and scanning electron microscopes indicate the foliicolous chlorophyte *Phycopeltis hawaiiensis* King (Chroolepidaceae) is subcuticular and polystromatic, produces sterile hairs, and induces a distinct wounding, reaction in subtending host tissue. In comparison, similar observations on *Phycopeltis epiphyton* and other *Phycopeltis* species reveal that, although they are also foliicolous, they are supracuticular and monostromatic. Also, they neither produce sterile hairs nor induce a wounding response in the host leaf. Because the observed characteristics of *Phycopeltis hawaiiensis* are similar to those of *Cephaleuros virescens* and other *Cephaleuros* species, it is suggested that *Phycopeltis hawaiiensis* is probably a *Cephaleuros* species; however, the absence of reproductive structures renders the designation of a specific epithet problematic.

IN THE FALL of 1973, Dr. Shirley C. Tucker of our department collected leaves of *Sabal minor* (Arecaceae) which bore a foliicolous supracuticular alga readily identifiable as *Phycopeltis* (and later identified by Dr. R. H. Thompson, University of Kansas, as *P. epiphyton*). Although *Phycopeltis* has been collected in the United States (King 1954; and R. H. Thompson, personal communication), there are few published accounts, and the only significant taxonomic reference at this time is a preliminary study of the "Trentepohliaceae" by Printz (1939). The descriptions and keys in this work were of limited usefulness in our attempts to identify with certainty the species of *Phycopeltis* that occur on *Sabal minor* and numerous other hosts (including *Arundinaria*, *Camellia*, *Cycas*, *Ilex*, *Ligustrum*, *Magnolia*, *Osmanthus*, *Podocarpus* and *Quercus*) in Baton Rouge and environs. Because *Phycopeltis hawaiiensis* King (Chroolepidaceae)[4] was not included in Printz's work and because the original description of *P. hawaiiensis* (King 1954) was limited and not accompanied by drawings or photographs of the alga in its natural (i.e., foliicolous) condition, the holotype specimen was obtained for comparison with the Louisiana material. The size and general appearance of *P. hawaiiensis* was unlike that of *P. epiphyton*, which epiphytizes numerous vascular plants in Louisiana, and unlike other species of *Phycopeltis* which we had examined; therefore, samples of *P. hawaiiensis* were prepared for light and scanning electron microscopic examination in order for us to determine whether or not the alga was *Phycopeltis*. For comparison, samples of *P. epiphyton* and *Cephaleuros virescens* (a related epiphytic and parasitic alga which is being studied in our laboratory [Chapman 1974, 1975, 1976*a*, *b*; Chapman and Madison 1975; Good and Chapman 1975, in press]) were also prepared for light and/or scanning electron microscopic observation.

MATERIALS AND METHODS

Light Microscopy

Material prepared included *Phycopeltis hawaiiensis* foliicolous on *Schefflera* sp.; *P. epiphyton*, on *Sabal minor*; and *Cephaleuros virescens*, on *Magnolia grandiflora*. The latter specimens were collected and fixed shortly thereafter without preliminary treatment; the *Phycopeltis hawaiiensis* was prepared prior to fixation as follows: small portions of a leaflet bearing the alga were

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[4] As Papenfuss (1962) has noted, Chroolepidaceae has priority over the more commonly used Trentepohliaceae.

excised from the herbarium specimen, placed in a moist chamber, and maintained at about 27° C for 10 days. All material was fixed in Formalin–acetic acid–ethanol (1:1:18) or chromate–acetic acid–Formalin III for 24 hours, softened in hydrofluoric acid (1:1 aqueous dilution) for about 110 hours, washed for 12 hours, dehydrated in a graded tertiary butyl alcohol series, and embedded in paraffin. Eight μm sections were produced with a Spencer 820 rotary microtome (American Optical Corporation), stained (safranin-fast green, hematoxylin, or tannic acid schedules), and examined and photographed with a Leitz Orthoplan microscope with Orthomat camera.

Scanning Electron Microscopy

Small samples of air-dried *Phycopeltis hawaiiensis*, *P. epiphyton*, and *Cephaleuros virescens* (on *Schefflera* sp., *Sabal minor*, and *Magnolia grandiflora*, respectively) were mounted with silver conducting paint, on aluminum stubs, coated *in vacuo* with a thin (about 20 nm) layer of gold, and examined in a Jeolco JSM-2 scanning electron microscope (SEM), operated at 25 kv. Comparable specimens of freshly collected *P. epiphyton* and *C. virescens* were fixed with 3.5 percent glutaraldehyde in 0.02 *M* phosphate buffer (*p*H 6.8) for 2.5 hours at 23° C, rinsed with buffer, dehydrated in an ethanol-amyl acetate series, and dried with CO_2 in a Denton DCP-1 critical-point dryer. Critical-point dried specimens were mounted and coated as described above.

OBSERVATIONS

General

Examination of the holotype specimen of *Phycopeltis hawaiiensis* (Figure 1) revealed from 50 to over 150 macroscopic algal "spots" on each leaflet. The spots range in morphology from small (1–5 mm in diameter) round protuberances (individual thalli) to larger (5 × 12 mm) irregular aggregations formed from the merger of two or more thalli (Figure 2).

Light Microscopy

Observations made with a dissecting microscope indicate that *P. hawaiiensis* thalli bear sterile hairs singly or in clusters, but do not bear sporangiophores (or "sporangiate branches"). The edges of algal disks closely adhered to the leaf surface and no loose or upturned edges were observed. Small thalli (less than 1 mm in diameter) were easily pushed off the leaf surface with a dissecting needle; however, larger disks were very firmly attached and could not be removed from the leaf surface without damage to the leaf.

Three principal observations were made on sectioned *P. hawaiiensis*. First, medium- and large-sized thalli (such as the medium-sized disk about 5 mm in diameter, shown in Figure 3) are subcuticular (Figure 3, arrows); second, a massive wound parenchyma is present in the host tissue subtending the alga (Figure 3) but is absent in other areas lacking the alga (Figure 4); and, third, rhizoidal filaments are produced along the ventral surface of the alga. *Cephaleuros virescens* on *Magnolia grandiflora* grows beneath the cuticle, induces a wounding response in subtending host tissue, and produces rhizoidal filaments (Figure 5). *Phycopeltis epiphyton* on a leaf of *Sabal minor* is supracuticular, does not evoke a wounding response in the host, and is completely monostromatic, i.e., lacks any rhizoidal filaments (Figure 6).

Scanning Electron Microscopy

As seen with the SEM, the relatively smooth surface of *P. hawaiiensis* does not reveal a pattern of cell arrangement, whereas in *P. epiphyton* (Figure 7) and *Phycopeltis* sp. (Figure 8) the cell arrangement is readily observable in both air-dried and critical-point dried material. Further, the edges of each *P. hawaiiensis* thallus are not visible in SEM preparations; rather, at the thallus margin there is a smooth continuous slope which is similar to that which occurs in both air-dried and critical-point dried *Cephaleuros virescens* samples. In the latter the distinct edge of each disk is closely appressed to the epidermis beneath the cuticle and hence is not directly observable with the SEM. In the case of *P. epiphyton* and other species, the disk edges are not only directly observable but are often raised above the surface of the leaf (Figures 7 and 8). Scanning electron microscopic observations on the sterile hairs, which arise from the *P. hawaiiensis* thallus and emerge through the host cuticle (Figure 9), are quite similar to our observations on air-dried *Cephaleuros* but are unlike our observations on *Phycopeltis* spp.

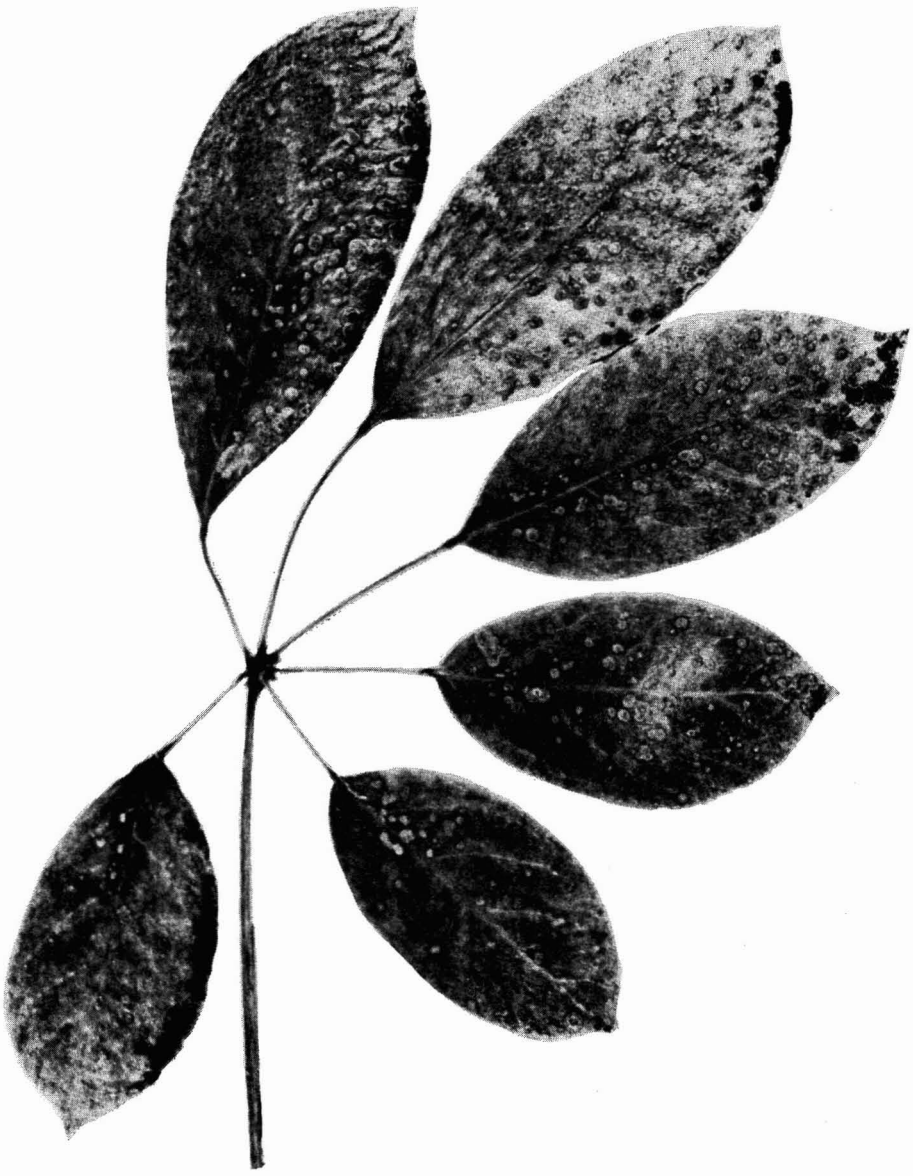

FIGURE 1. *Phycopeltis hawaiiensis* King on *Schefflera* (holotype), approximately ×0.5.

DISCUSSION

The dissimilarity between the general appearance of *Phycopeltis hawaiiensis* on the one hand and several other *Phycopeltis* species on the other prompted us to question the generic placement of the plant. The smaller disks (1–5 mm diameter) of *P. hawaiiensis* are larger than expected for many species of *Phycopeltis* (Cribb 1967, Karsten 1891, Printz 1939). And, although individual disks of *Phycopeltis* do merge and can form sheets, the general appearance of such forms is quite unlike that of *P. hawaiiensis*. The general appearance of

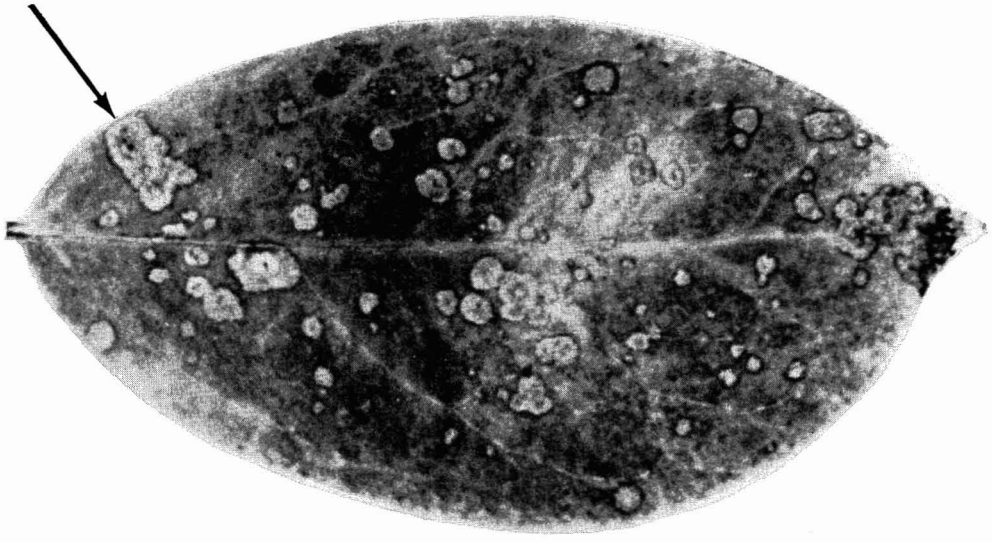

FIGURE 2. A leaflet from Figure 1 enlarged to show numerous *P. hawaiiensis* thalli, including several which have merged (e.g., at arrow), approximately ×1.25.

P. hawaiiensis differs markedly not only from that of *P. epiphyton* which occurs in Louisiana but also from that of *P. arundinacea*, *P. irregularis*, and *P. treubii* (University of California at Berkeley herbarium specimens 763927, M 020266 and 761292–3, respectively). Similarly a comparison between *P. hawaiiensis* and other *Phycopeltis* species described and illustrated by Printz (1939) reveals major differences. In contrast, however, *P. hawaiiensis* is similar in general appearance (i.e., size and distribution on the leaf surface) to *Cephaleuros virescens* foliicolous on *Magnolia grandiflora* and other hosts. Perhaps more significant than the observed differences in appearance are the observations that *P. hawaiiensis* grows subcuticularly on *Schefflera*, produces rhizhoidal processes, and evokes a wounding response in the subtending host tissue. These observations indicate that *P. hawaiiensis* is probably a *Cephaleuros* species (Joubert and Rijkenberg 1971; Karsten 1891; Printz 1939, 1964; Thomas 1913; R. H. Thompson, personal communication; Wolf 1930). Also, the presence of sterile trichomes arising from the *P. hawaiiensis* thalli supports the suggestion that the organism is a *Cephaleuros* species rather than a *Phycopeltis* species. Although the genus *Phycopeltis* could be emended to include such an atypical species, such a change for *P. hawaiiensis* is unwarranted, especially in view of the fact that the original material is sterile and cannot be identified to the species level (R. H. Thompson, personal communication).

It should be mentioned that, although both *P. hawaiiensis* on *Schefflera* and *Cephaleuros virescens* on *Magnolia grandiflora* induce a wound response in subtending host tissues, the type of response is different. In the former, there is a corklike region of vertically storied rectangular cells produced by a phellogen presumably originating from the cells of the upper hypodermis. In the latter, there are numerous abnormal cell divisions in the subepidermal parenchyma as well as massive chemical change (suberin deposition?) in the parenchyma and epidermis. That the wounding responses are dissimilar is not unexpected since the nature and severity of wounding responses to different species of *Cephaleuros* is determined by several factors, including the specific alga, the specific host, and the physiological condition of the host. The host wounding response is, in fact, an interesting aspect of the biology of *Cephaleuros* which warrants further study.

It should be noted that King (1954, 1955)

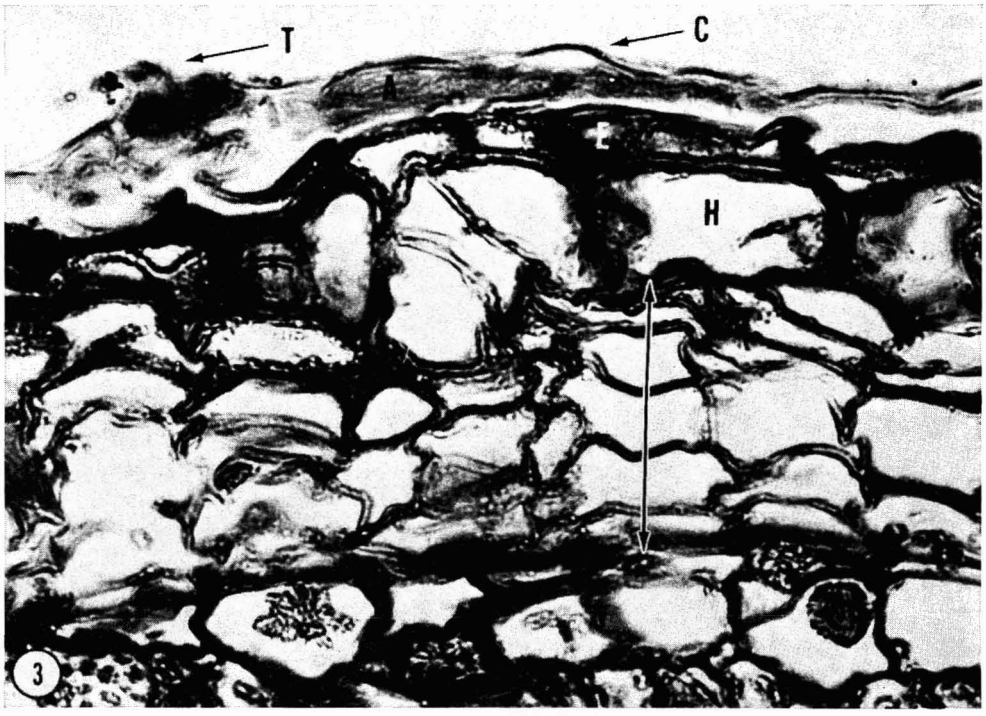

FIGURE 3. *P. hawaiiensis* on *Schefflera* (from holotype) cross-sectional view, the algal thallus (A) below the host cuticle (C) with the base of a sterile trichome (T) in the plane of section. Note region of wound parenchyma (indicated by the double-headed arrow) occurring beneath epidermis (E) and hypodermis (H). Compare with Figure 4. ×630.

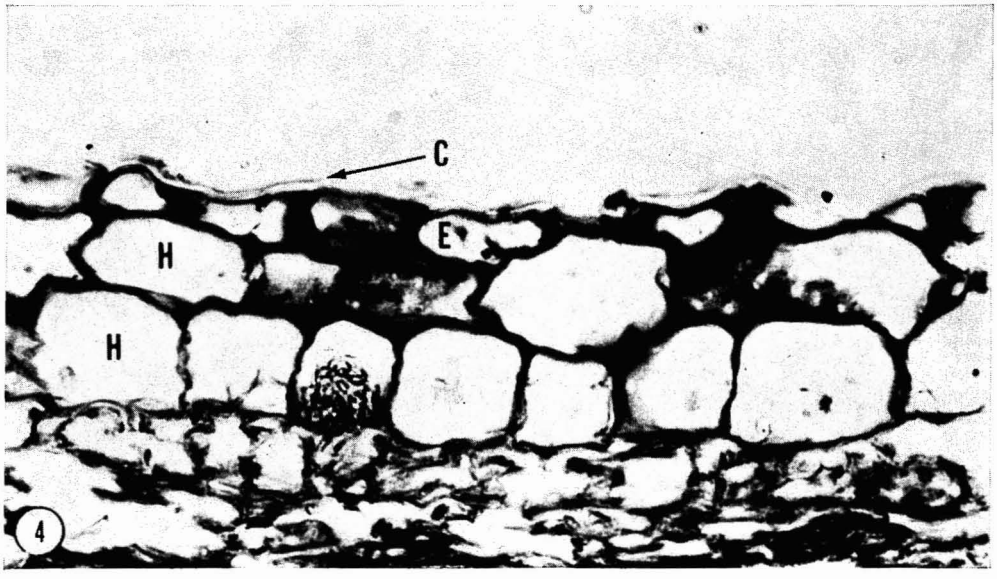

FIGURE 4. Normal *Schefflera* leaflet (from holotype), ×5, showing cuticle, epidermis, and hypodermis. ×630.

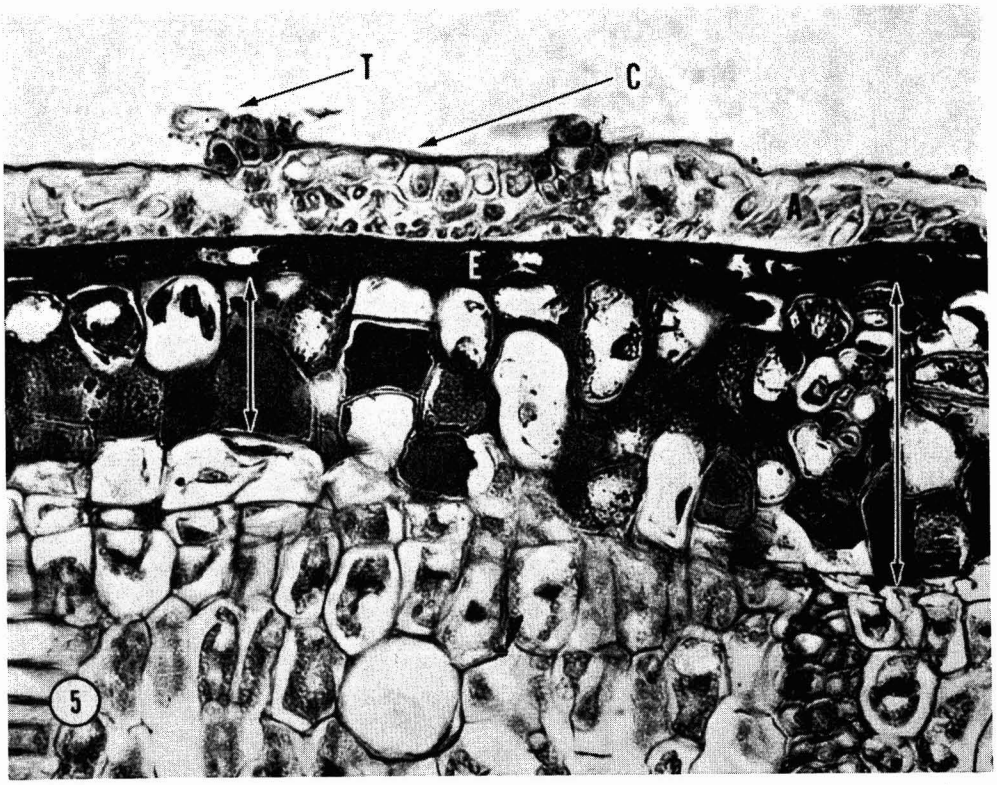

FIGURE 5. *Cephaleuros virescens* foliicolous on *Magnolia grandiflora* occurs beneath host cuticle (C), produces trichomes (T), and induces a massive wound response (indicated by double-headed arrows and differential staining) beneath epidermis (E). ×370.

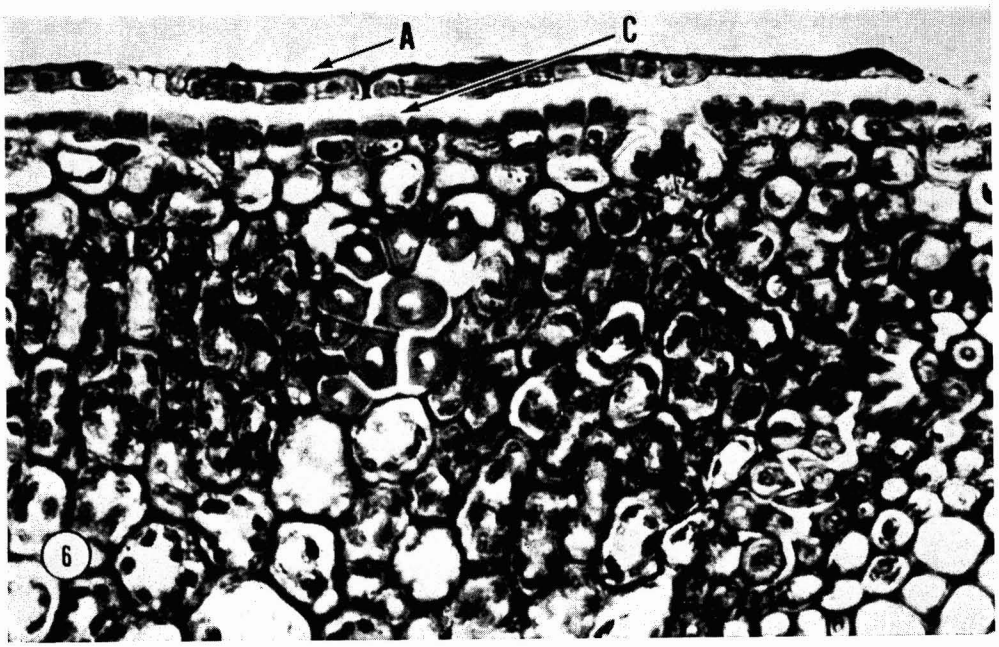

FIGURE 6. *Phycopeltis epiphyton* foliicolous on *Sabal minor* growing above the cuticle (C) may actually be raised above the leaf surface (note space between algal thallus (A) and cuticle; also see Figures 7 and 8). Subtending host tissue appears normal and lacks wound tissue. ×350.

FIGURE 7. *Phycopeltis epiphyton* foliicolous on *Schefflera minor* showing cellular pattern and distinct thallus margins (SEM preparation). ×350.

FIGURE 8. *Phycopeltis* sp. foliicolous on *Arundinaria*. Note a portion of one thallus above leaf surface (SEM preparation). ×1020.

FIGURE 9. Sterile trichomes of *P. hawaiiensis* emerging through host cuticle (SEM preparation). ×900.

studied "haematochrome" accumulation in culture-grown *P. hawaiiensis* and published a light micrograph of the culture-grown alga (King 1954). In general appearance, his culture-grown material is similar to culture-grown *Cephaleuros virescens* which we have observed in our laboratory; hence, neither King's experiments nor the light micrograph conflict with the suggestion that the alga is *Cephaleuros* and not *Phycopeltis*. It is not unreasonable to suggest that the phenomenon of "haematochrome" accumulation in the two genera might be similar; but, even if it is not, the fact that the experiments may have been conducted on *Cephaleuros*, not *Phycopeltis*, does not alter King's results.

Despite the close association of a fungus with *P. hawaiiensis*, the possibility that *P. hawaiiensis* is actually a lichenized alga was not accepted by King (1954) and seems unlikely to us, since neither reproductive structures nor other indications of lichenization have been observed. Although lichenization of *Cephaleuros* does occur in Louisiana, fungi are frequently associated with the free-living alga as well and a similar situation presumably occurred in the case of *P. hawaiiensis*.

Our observations indicate that *Phycopeltis hawaiiensis* is probably a *Cephaleuros* species, but they are not sufficient for the transfer or assignment of a specific epithet. Certainly both the sterile condition of the original material and the limited description presented by King (1954) necessitate additional collections and further study of this *Cephaleuros*.

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LITERATURE CITED

CHAPMAN, R. L. 1974. Observations on the ultrastructure and taxonomic position of the green alga *Cephaleuros virescens*. Proc. La. Acad. Sci. 37: 130.

———. 1975. Preliminary electron microscopic observations on lichenized *Cephaleuros* (*Strigula*). Tex. Rep. Biol. Med. 33: 346–347.

———. 1976*a*. Ultrastructural investigation on the foliicolous pyrenocarpous lichen *Strigula elegans* (Fée). Müll.Arg. 15: 191–196.

———. 1976*b*. Ultrastructure of *Cephaleuros virescens* (Chroolepidaceae; Chlorophyta). I. Scanning electron microscopy of zoosporangia. Amer. J. Bot. 63: 1060–1070.

CHAPMAN, R. L., and S. W. MADISON. 1975. Scanning and transmission electron microscopic observations on the development and detachment of terminal sporangia in Cephaleuros virescens (Trentepohliales). ASB Bull. 22: 45–46.

CRIBB, A. B. 1967. *Phycopeltis kosteriana* sp. n., an epiphyllous alga of the family Chroolepidaceae from Australia. Blumea 15(1): 3–4.

GOOD, B. H., and R. L. CHAPMAN. 1975. Preliminary observations on the epiphytic green algae *Phycopeltis epiphyton* and *Phycopeltis hawaiiensis* (Trentepohliaceae). Proc. La. Acad. Sci. 38: 122, pls. 76*a*, 76b.

———. In press. Initial microscopic observations on species of *Phycopeltis* (Chlorophyta, Chroolepidaceae). Proc. La. Acad. Sci.

JOUBERT, J. J., and F. H. J. RIJKENBERG. 1971. Parasitic green algae. Annu. Rev. Phytopathol. 9: 45–65.

KARSTEN, G. 1891. Untersuchungen ueber die Familie der Chroolepideen. Ann. Jard. Bot. Buitenzorg 10: 1–66.

KING, J. W. 1954. An investigation of hematochrome accumulation in the alga *Phycopeltis hawaiiensis* n.sp. P.ac. Sci. 8(2): 205–208, 2 pls.

———. 1955. Further investigation on hematochrome accumulation in the alga *Phycopeltis hawaiiensis*. Biol. Bull. (Woods Hole, Mass.) 109(3): 361–362.

PAPENFUSS, G. F. 1962. On the circumscription of the green algal genera *Ulvella* and *Pilinia*. Phykos 1: 6–12.

PRINTZ, H. 1939. Vorarbeiten zur einer Monographie der Trentepohliaceen. Nytt Mag. Naturv. 80: 137–210.

———. 1964. Die Chaetophoralen der Binnengewässer: eine systematische Übersicht. Hydrobiologia 24: 1–276.

THOMAS, N. 1913. Notes on Cephaleuros. Ann. Bot. (London) 27: 781–797.

WOLF, F. A. 1930. A parasitic alga, *Cephaleuros virescens* Kunze, on *Citrus* and certain other plants. Elisha Mitchell Sci. Soc. J. (Chapel Hill, North Carolina) 45: 187–205.